REMARKS

This Reply is responsive to the Office Action dated October 19, 2001. Entry and consideration of the amendments and remarks submitted herein pursuant to 37 C.F.R. §1.116 is respectfully requested.

At the outset, applicants note that the claims have been amended to reflect the elected invention, which was limited to the species of *in vivo* maintenance of stem cells with a further species election of providing a nucleic acid encoding Dpp to accomplish the claimed methods. However, applicants respectfully request rejoinder of non-elected subject matter upon the indication of allowable claims, including that subject matter that would not raise further issues with regard to patentability. In particular, applicants believe that it would be appropriate to rejoin the use of other BMP derivatives upon allowance. According to the Office Action at page 2, claims 3, 11-15, 17, 19-22, 32 and 34 were withdrawn from further consideration because they are drawn to a non-elected invention, leaving claims 1, 2, 4-10, 16, 18, 23-31, 33, 35 and 36 as being currently under examination.

Applicants respectfully request reconsideration of the withdrawal of claims 12, 13 and 17, as separation of these claims from the claims currently undergoing examination appears to have been in error. Claim 12 recites that the BMP (Dpp) signaling pathway is stimulated "through" at least one receptor that recognizes Dpp. Claim 13 recites several receptors that are known to recognize Dpp, as disclosed in the specification at page 3 starting at line 16, to page 4, line 7. Thus, claims 12 and 13 further define a portion of the Dpp signaling pathway that is active upon expression of a nucleic acid encoding Dpp, that being the recognition of the expressed Dpp

protein by Dpp-specific receptors. Thus, the invention recited in claims 12 and 13 is not different from the elected invention, and should therefore be included in the claims undergoing examination.

Similarly, claim 17 is directed to a method according to claim 1 wherein BMP (Dpp) expression is increased by *hedgehog*- or *wingless*-activated transcription. Thus claim 17 recites one method of providing for expression of a nucleic acid encoding Dpp as discussed at the paragraph bridging pages 15 and 16 and as elected in the present application, and should therefore be included in the claims currently undergoing examination.

Besides being amended to reflect the elected invention, claim 1 was amended to clarify that it is a germline cell in the recited population of cells in which the recited BMP signaling pathway is stimulated. Claims 1, 33, 35 and 36 were also amended to clarify that the recited signaling "increases the abundance" of the germline stem cells in the population or organism as compared to a population or organism in which signal transduction of a BMP signaling pathway has not been stimulated. Support for such language may be found at the very least in original claim 35, and at the specification at page 4, line 30. Applicants believe that this amendment merely clarifies what was already evident in the claim as originally submitted, given that original claim 1 referred to "maintaining more" germline cells in the stimulated population as compared to an unstimulated population. Applicants believe that claim 1 as amended would still encompass the maintenance of "more" germline cells once their abundance has been increased as argued in applicants' previous Reply (filed August 14, 2001, at page 2).

Claim 2 was amended to delete reference to *in vivo* maintenance given the amendment to

claim 1 to reflect the elected invention.

Claim 7 was amended to delete unnecessary verbiage and to maintain consistency with claim 1 on which it depends.

Claims 9 and 10 were canceled because the limitations recited therein were incorporated into claim 1 to reflect the elected invention.

Claims 12 and 13 were amended to specify that the claimed receptor specifically recognizes Dpp rather than a BMP in general to reflect the elected invention, in the anticipation that claims 12 and 13 will be joined with the elected invention.

Claims 16 and 17 were amended to specify that expression of Dpp is increased rather than expression of a BMP in general, again to reflect the elected invention and in the anticipation that claim 17 will be joined with the elected invention.

Claims 25-27 were amended to qualify the host Drosophila recited therein as the "second" host Drosophila in view of the amendment to claim 1 in accordance with the elected invention.

In addition, new claims 37-43 were added. Claim 37 is directed to the method according to Claim 1, wherein there are at least ten germline stem cells in said population for each stem cell present prior to stimulation of BMP signaling. Support for this claim may be found in original claim 32.

Claims 38 and 39 are directed to the method of claim 1, wherein said host Drosophila contains at least a hundred, or at least five hundred germline stem cells, respectively, following stimulation of said BMP signaling pathway. Support for these claims may be found at page 17,

lines 7-11. For instance, this passage discloses that the wildtype number of stem cells per ovariole is two or three (line 7). Given that there are 16 ovarioles per ovary and two ovaries per female fly (lines 9-11), at the most there are 3 X 32 germline stem cells, or 96 germline stem cells in a wildtype female fly.

In contrast, in the flies of the present invention, there were dozens of germline stem cells in a single ovariole (page 17, line 8). Taking the lowest number disclosed, i.e., a dozen or twelve per ovariole, a fly produced according to the methods of the present invention would have 12 X 32 germline stem cells, or 384 germline stem cells. Even taking the disclosed "dozens" as meaning just two dozen germline stem cells per ovariole, such a fly would have 24 X 32 or 768 germline stem cells. Moreover, the observed number was 2-3 times greater after 4-5 days than after 3 days (lines 8-9). Indeed, the present inventors have found that flies produced according to the invention wherein excess Dpp protein is provided contain hundreds and even thousands of germline cells. Thus the numbers as recited in new claims 38 and 39 find support at page 17, lines 7-11 and would be recognized as being implicitly disclosed by a person of skill in the art reading the specification.

New claim 40 is directed to the method of claim 1 and further defines the nucleic acid expression system used to provide Dpp. Support for this claim may be found at page 16 in Example 1.

New claim 41 is directed to the method of claim 1 and specifies that the method may include a further step of isolating the germline stem cells produced by the claimed *in vivo* methodology for subsequent *in vitro* culture. Support for this claim may be found at page 12, lines 16-21.

New claims 42 and 43 specify that the recited increase in germline stem cells as recited in Claim 1 may be further supplemented by altering the activity of downstream Dpp signal

transducing proteins. For instance, such further manipulation may include decreasing the concentration of downstream negative regulators, e.g., the Dad gene product, or by overexpressing or expressing modified Mad or Madea, positively activing transcription factors that transduce the dpp signal, or Schnurri, another downstream factor in the pathway that has been shown to act on germ line stem cells. Support for these claims may be found in original claims 14 and 15.

No new matter has been added by any of the amendments or new claims submitted above.

Turning now to the Office Action, claims 1, 2, 4-10, 16, 18, 23-31, 33, 35 and 36 remain rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness. Specifically, claim 1 is alleged to be vague in the recitation of stimulating a BMP signaling pathway in at least one cell of the population because it is not clear whether it is the germline cell in the population in which signaling occurs. Applicants have amended claim 1 to clarify that signaling occurs in a germline cell of the population, therefore, this rejection is now moot.

Claim 7 was alleged to be vague in the recitation of providing at least 10% more Dpp activity because the type of Dpp activity is not defined, because the claim language encompasses the use of mutant alleles whose activity is not defined, and because it is not clear precisely what is being stimulated. Applicants respectively maintain their traversal of the rejection of claim 7.

First, applicants fail to understand why the term "stimulated" is unclear in the context of claim 7 when claim 7 employs the same context as claim 1 on which it depends. Indeed, both claims specify that a BMP signaling pathway is stimulated, so it is not clear why claim 7 is less clear in this regard than claim 1. Nevertheless, in an abundance of caution, claim 7 has been amended to delete the language that is alleged to be vague because this language is unnecessary in any case.

Claim 7 was also amended above to delete the word "activity," as the recitation of this word appears to be causing needless confusion. Amended claim 7 is now directed to the method of claim 1 in which at least 10% more Dpp protein is provided to the cell population than in wildtype Drosophila (without referring to the stimulation of a BMP signaling pathway). Increased Dpp protein may be provided to the cell population in several ways, many of which are recited in the other dependent claims. For instance, as recited in claims 16 and 17, the method may be accomplished by increasing the expression of *dpp*, *i.e.*, endogenous *dpp*, because increasing the expression of *dpp* provides a nucleic acid encoding Dpp so as to meet the requirements of the claim. As recited in pending claim 8, *dpp* expression may be provided by mutating a *dpp* gene to a gain-of-function phenotype thereby leading to greater signaling activity. Alternatively, as recited in new claim 40 and covered generically by original claim 2, Dpp may be provided by expressing it ectopically in germarium of the host Drosophila using hsp70-GAL4 and UAS-*dpp* or any other *dpp* transgene. Therefore, amended claim 7 is clear and meets the requirements of §112, second paragraph.

Claim 10 was rejected under §112, second paragraph, for reciting other BMP species not elected for examination. Claim 10 was canceled above, so this rejection is moot.

Claim 26 was rejected under §112, second paragraph, because the further step of differentiating the transferred stem cells in a second host Drosophila removes the invention from the original scope set forth in the preamble of the independent claim 1. The Examiner suggested that claim 26 be amended to indicate that differentiation is an inherent property of the germline cells of the method, rather than have it be an active step of the method. Applicants have adopted the Examiner's suggestion, and therefore, the rejection of claim 26 under §112, second paragraph is moot.

Claims 1, 2, 4-10, 16, 18, 23, 24, 28-31, 33, 35 and 36 were rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Twombley *et al.* Essentially, it is the Examiner's position that, because Twombley *et al.* teach expression of a *dpp* transgene in Drosophila, the method of Twombley would inherently result in increased maintenance of the germline stem cells as recited in the instant claims. Applicants respectfully maintain their traversal.

First, applicants respectfully note that the independent claims 1, 33, 35 and 36 were amended above to clarify that stimulation of the BMP signal transduction according to the invention by expressing a nucleic acid encoding Dpp results in an increased abundance of germline stem cells as compared to a population or organism in which the BMP signal transduction is not stimulated. Although the Examiner is correct to note that Twombley expresses a *dpp* transgene and therefore may achieve maintenance of stem cells similar to that inherently achieved in a wildtype population or Drosophila, the mere expressing of a *dpp* transgene would not be expected to result in an increased abundance of stem cells relative to a wild type or unstimulated population as disclosed in the present specification and recited in the amended claims.

Indeed, Twombley *et al.* expressed a conditional partial loss-of-function *dpp* allele (dpp^{ea87}/dpp^{hr56}) in Drosophila so that they could control the timing of *dpp* expression in order to examine the role of Dpp in maintaining egg chamber integrity. Twombley says nothing of an increased abundance of stem cells being produced by this expression of a *dpp* transgene, which is consistent with their goal of controlling the timing of expression of *dpp*, rather than over-expressing *dpp* or expressing it in a manner or at a time that increases the abundance of germline stem cells. In fact, applicants describe a similar set of experiments in Example 4 of the specification, where *dpp* expression was controlled by using temperature sensitive allelic combinations of *dpp* in order to examine the role of Dpp in germarium by abolishing its expression in adult Drosophila. These experiments were quite different than the over-expression of *dpp* as achieved in Example 1 of the specification. Likewise, Twombley *et al.* merely replaced wildtype expression of *dpp* with a transgene whose expression could be controlled, and studied the role of the gene by turning it off at various stages during oogenesis.

In contrast, applicants provide a nucleic acid encoding the Dpp protein and which is capable of expressing the protein at high enough levels at the appropriate time upon induction of gene expression so as to lead to an increase in abundance of germline stem cells. As a result of expressing Dpp in this manner, applicants made the novel discovery that increasing the level of *dpp* expression or the presence of Dpp protein may be used to produce Drosophila with significantly enhanced numbers of germline stem cells (see the specification at pages 4-5 and 16-

17). The prior art methods of expressing *dpp* transgenes do not "inherently" lead to such a method because these transgenes were not expressed in such a way so as to lead to an increase in the abundance of germline stem cells. Furthermore, it would not have been obvious to express a *dpp* gene or transgene in a manner so as to achieve such a goal without the knowledge of applicants' invention in hand.

Applicants have surprisingly found that Dpp expression may be controlled in such a way so as to produce greatly increased numbers of stem cells. For instance, as described on page 17, lines 7-11, while wildtype female flies generally have 2-3 germline stem cells per ovariole, the flies produced according to the present invention have dozens of stem cells per ovariole. In fact, the flies of the present invention have hundreds and even thousands of germline stem cells total, whereas wildtype female flies generally have no more than 96 total, given that there are 16 ovarioles. Such increased numbers are reflected in new claims 37-40, which are clearly not taught nor rendered obvious by the disclosure of Twombley.

Applicants' discovery has significant utility in that the claimed methods provide for a maintenance and culture system for valuable stem cells, which may then be isolated from flies produced according to the invention and used for further purposes. New claim 41 adds a further step to the claimed methods in this regard, whereby the increased numbers of stem cells produced by the method of claim 1 may be isolated and further cultured *in vitro*. Twombley neither discloses nor renders obvious the production or isolation of increased numbers of germline stem cells for subsequent *in vitro* culturing.

Thus, Twombley *et al.* do not inherently teach a method of increasing the abundance of stem cells *in vivo* in Drosophila as recited in the instant independent claims 1, 33, 35 and 36. Because dependent claims incorporate all the limitations of the base claim on which they depend, Twombley also fails to inherently teach any of the methods recited in claims 2, 4-8, 16, 18, 23, 24 and 28-31. Reconsideration and withdrawal of the rejection under 35 U.S.C. §102(b) based on Twombley are respectfully requested.

Claims 1, 2, 4-10, 16, 18, 23, 24, 28-31, 33, 35 and 36 were also rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Forbes *et al.* Essentially, it is again the Examiner's

position that, because Forbes *et al.* teach expression of a *dpp* transgene in Drosophila, the method of Forbes would inherently result in increased maintenance of the germline stem cells as recited in the instant claims. The Examiner also observes that Forbes *et al.* teach egg chamber structures containing 16 germ line cells whereas the germarium of the fly generally contains 2-3 germline cells. Thus, the Examiner believes that the expression of the *dpp* transgene in Forbes actually results in an increased number of germline cells. Applicants respectfully maintain their traversal.

First, applicants again respectfully note that the independent claims 1, 33, 35 and 36 were amended above to clarify that stimulation of the BMP signal transduction by expressing a nucleic acid encoding Dpp results in an increased abundance of germline stem cells as compared to a population or organism in which the BMP signal transduction is not stimulated. Although the Examiner is correct to note that Forbes expresses a *dpp* transgene, Forbes does not express their *dpp* transgene in a manner that results in an increased abundance of stem cells relative to a wild type or unstimulated population as disclosed in the present specification and recited in the amended claims.

Indeed, the Examiner was correct to note that Forbes *et al.* observed the formation of egg chambers containing a multiplicity of 16 germline cells following ectopic expression of a *dpp* transgene. However, the Examiner makes an unfounded leap to assert that this observation means that the *dpp* transgene of Forbes had an effect on the number of germline stem cells. Rather, as described on page 3291 of Forbes *et al.*, col. 2, these authors isolated fused egg chambers containing several germline cysts within a single follicle. As described in the Background of Invention, at page 2, lines 3-6, the cells in cysts are actually differentiated daughter cells of germline cells, which undergo four rounds of synchronous division to eventually produce 16 interconnected "cystocytes," the precursors of ovarian follicles. Further, as explained in Example 2 of the present specification, cystoblasts and early mitotic cells can be distinguished from stem cells in that the former express the cytoplasmic Bam protein whereas germline stem cells do not. Thus, Forbes *et al.* actually observed the differentiation of germ line cells, rather than the maintenance of undifferentiated cells or the increase of such cells as required by the present invention.

Furthermore, the present inventors show in fact that Dpp signaling is not involved in regulating cystoblast and cystocyte divisions at page 23, lines 19-24, because Drosophila mutants containing mutations in genes involved downstream in *dpp* signaling still produced cysts containing 16 cells. Thus, although Forbes *et al.* observed the fusion of egg chambers containing germline cysts upon ectopic expression of *dpp* transgene, Dpp expression was likely not responsible for the formation of the cysts observed. And in any case, fusion of egg chambers containing cystocytes does not equate to an increase in abundance of stem cells as required by the present claims. Rather, it equates merely to a reorganization (not even an increase) of cystocytes that are actually different than germline stem cells. Thus, although Forbes *et al.* expressed a *dpp* transgene ectopically in Drosophila, they did not express it or induce its expression in a manner sufficient to produce an increase in the abundance of stem cells.

In contrast, applicants expressed a *dpp* transgene at high enough levels and at just the right time during oogenesis so as to lead to an increase in abundance of germline stem cells. As a result of their Dpp expression system, applicants were able to make the novel discovery that controlling the level or timing of *dpp* expression may be used to produce Drosophila with significantly enhanced numbers of germline stem cells (see the specification at pages 4-5 and 16-17). The prior art methods of expressing *dpp* transgenes do not "inherently" lead to such a method because these transgenes were not expressed in such a way so as to lead to an increase in the abundance of germline stem cells. Furthermore, it would not have been obvious to express a *dpp* gene or transgene in a manner so as to achieve such a goal without the knowledge of applicants' invention in hand.

Thus, Forbes *et al.* do not inherently or explicitly teach a method of increasing the abundance of stem cells *in vivo* in Drosophila as recited in the instant independent claims 1, 33, 35 and 36. Because dependent claims incorporate all the limitations of the base claim on which they depend, Forbes *et al.* also fails to inherently or explicitly teach any of the methods recited in claims 2, 4-8, 16, 18, 23, 24 and 28-31. Reconsideration and withdrawal of the rejection under 35 U.S.C. §102(b) based on Forbes are respectfully requested.

Claims 1 and 25-27 were rejected under 35 U.S.C. §103(a) as being allegedly unpatentable

over either Forbes *et al.* or Twombley *et al.* and Lin *et al.* Essentially, it is the Examiner's position that Forbes *et al.* and Twombley *et al.* each teach expression of a *dpp* transgene that inherently results in an increase in germline stem cells, but that neither reference teaches the transfer of germline cells into a host Drosophila. However, the Examiner believes that such transfer would have been obvious in view of the disclosure of Lin *et al.*, who teach the transfer of germline stem cells into a host Drosophila for the study of germarial cells. Applicants respectfully maintain their traversal.

As explained above, neither Forbes nor Twombley teach expression of a *dpp* transgene so as to inherently result in an increase in stem cells. Heat shock-induced expression of the *dpp* transgene in Forbes *et al.* resulted in the formation of fused egg chambers containing germline cysts rather than germline stem cells. Thus, Forbes and colleagues did not express their *dpp* transgene in such a manner so as to achieve an increase in the number of stem cells.

Twombley *et al.* teaches the expression of a conditional partial loss-of-function *dpp* allele (dpp^{cs87}/dpp^{hr56}) in Drosophila so that they could control the timing of *dpp* expression in order to examine the role of Dpp in maintaining egg chamber integrity. Thus, Twombley *et al.* merely replaced wildtype expression of *dpp* with a transgene whose expression could be controlled, and studied the role of the gene by turning it off at various stages during oogenesis. Twombley says nothing of an increased abundance of stem cells being produced by this expression of a *dpp* transgene, which is consistent with their goal of controlling the timing of expression of *dpp*, rather than over-expressing *dpp* for the purpose of increasing the abundance of germline stem cells.

Thus, neither Forbes nor Twombley teach expression of a *dpp* transgene so as to inherently result in an increase in stem cells as required by claim 1. Lin *et al.* does not concern the expression of *dpp* and do not teach the explicit or inherent maintenance or increase in the number of germline stem cells in Drosophila. Thus, Lin *et al.* do not make up for the deficiencies of Forbes *et al.* and Twombley *et al.* as applied against the claim 1. Claims 25-27 are directly or indirectly dependent on claim 1 and therefore incorporate all its limitations. Therefore, neither Forbes *et al.* nor Twombley *et al.* nor Lin *et al.* is an effective reference against claims 25-27.

Further, there is nothing in the combination of these references that renders the claimed invention obvious, particularly since the Forbes *et al.* and Twombley *et al.* references cannot be relied upon for teaching an inherent increase in stem cells as alleged in the Office Action. Accordingly, reconsideration and withdrawal of the §103(a) rejection based on the combination of Forbes *et al.* or Twombley *et al.* and Lin *et al.* are respectfully requested.

CONCLUSION

This Reply is fully responsive to the Office Action dated October 19, 2001. Therefore, a Notice of Allowance is next in order. The Examiner is urged to contact the undersigned regarding any further issues or questions raised by this Reply, so that an allowance of the claimed subject matter may be expedited.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17, which may be required, to our Deposit Account No. 50-0310. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully Submitted,

Date: February 19, 2002

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APPENDIX

The following amendments were entered above:

IN THE CLAIMS:

Claims 9 and 10 were canceled.

The following claims were amended:

1. (Amended) A method for maintaining germline stem cells of Drosophila *in vivo* comprising:

   (c) providing a population comprised of said germline stem cells in a host Drosophila; and

   (d) stimulating signal transduction [by] of a bone morphogenetic protein (BMP) signaling pathway in at least one germline stem cell of said population by providing expression of a nucleic acid encoding Decapentaplegic (Dpp) protein;

   wherein said stimulation [maintains more] increases the abundance of germline stem cells in said population as compared to a population in which [has not had] signal transduction of said BMP signaling pathway has not been stimulated.

2. (Twice Amended) A method according to Claim 1, wherein said [population is maintained *in vivo* and a] Drosophila containing the germline stem cells has been genetically engineered to stimulate signal transduction.

7. (Amended) A method according to Claim 1, wherein [said BMP signaling pathway is

stimulated by providing] at least 10% more Decapentaplegic (Dpp) [activity] protein is provided to said population than is present in wildtype Drosophila.

12. (Amended) A method according to Claim 1, wherein said BMP signaling pathway is stimulated through at least one serine/threonine kinase receptor that specifically recognizes [a BMP] said Dpp.

13. (Amended) A method according to Claim 12, wherein said [BMP] Dpp receptor is selected from the group consisting of Saxophone (Sax), Thick veins (Thv), and Punt (Put).

16. (Amended) A method according to Claim 1, wherein said BMP signaling pathway is stimulated by increasing expression of [BMP] Dpp in a cell of said population.

17. (Amended) A method according to Claim 16, wherein [BMP] Dpp expression is increased by *hedgehog* (*hh*)-activated transcription or *wingless* (*wg*)-activated transcription, and [BMP] Dpp signaling is increased in at least some of the germline stem cells.

25. (Amended) A method according to Claim 1 further comprising transferring at least one of said stimulated germ line stem cells into a second host Drosophila.

26. (Amended) A method according to Claim 25, wherein at least one of said transferred germline stem cells is capable of contributing [contributes] to two or more differentiated cell lineages of said second host Drosophila.

27. (Amended) A method according to Claim 25, wherein at least one of said transferred germline stem cells contributes to a germline cell lineage of said second [host] Drosophila.

33. (Amended) A method for maintaining Drosophila stem cells *in vivo* comprising:

(c) providing a population comprised of said stem cells, and

(d) stimulating a *decapentaplegic* (*dpp*) signaling pathway in at least one stem cell in said population by providing expression of a nucleic acid encoding Dpp,

such that [more stem cells of said population are maintained as at least viable or] there is an increase in abundance of undifferentiated stem cells in said population as compared to a population of stem cells which has not been stimulated.

35. (Amended) A method of increasing abundance of stem cells of an organism *in vivo* comprising:

stimulating signal transduction by a bone morphogenetic protein (BMP) receptor

pathway by providing expression of a nucleic acid encoding Dpp such that abundance of at least some stem cells is increased as compared to an organism in which BMP signal transduction is not stimulated.

36. (Amended) A method of increasing lifetime of stem cells of an organism *in vivo* comprising:

stimulating signal transduction by a bone morphogenetic protein (BMP) receptor pathway by providing expression of a nucleic acid encoding Dpp such that said lifetime of at least some stem cells is increased, wherein said increased lifetime of at least some stem cells leads to an increased abundance of stem cells in said organism as compared to an organism in which BMP signal transduction is not stimulated.